

ABSTRACT

One of the most important and widespread problems faced by dairy cattle breeders is udder infections. *Mastitis*, defined as inflammation of the mammary gland, is a disease whose varied underlying causes, severity, and impact on the animal's physiological state depend on the environment, the pathogenic factor, and the host organism. Milk from infected quarters of the udder is unsuitable for consumption, which often leads to significant financial losses incurred by the dairy industry. One way to reduce the incidence of *mastitis* is to use genetic differences in susceptibility to this condition in breeding work. Research is ongoing to identify marker genes associated with *mastitis*. Single nucleotide polymorphisms (SNPs) in genes related to the immune response can be a useful marker for early detection of *mastitis* in dairy cattle.

The aim of this study was to find associations between immunity/susceptibility to *mastitis* (expressed as somatic cell count (SCC) in milk), daily milk yield, as well as the percentage of protein and fat in milk, and genetic variants of genes involved in immune processes - *microRNA2467*, *microRNA2313*, *microRNA2489*, *microRNA29b-2*, and *microRNA29e*.

The study involved a herd of 644 Polish Holstein-Friesian cows of the black-and-white variety. Results from 22 292 milk yield samples were collected from breeding records and included daily milk yield, percentage of protein and fat, and SCC. Peripheral blood was taken from all animals studied, and genomic DNA was isolated. Polymorphism of the *microRNA* genes studied - *microRNA2467*, *microRNA2313*, *microRNA2489*, *microRNA29b-2*, and *microRNA29e* - was identified by PCR-RFLP method. The studied cows were in lactation from the first to the eighth. The analyses were conducted separately for each lactation, and only due to the relatively small number of cows in later lactations, lactations 6th, 7th; and 8th were combined into one class. The effects of the selected genes were also studied in three successive lactation stages. In addition, the milk samples studied were divided into three classes of somatic cell count values: healthy cows with $SCC \leq 200$ thousand/ml, subclinical state $200 < SCC \leq 400$ thousand/ml, and sick cows with $SCC > 400$ thousand/ml. This division was made due to the possibility of different effects of the studied genes in different physiological states.

The obtained results were analyzed and subjected to statistical analysis. The genetic structure of the studied population was determined, i.e., the frequencies of alleles and genotypes of the analyzed genes. The statistical analysis of the results also included the search for associations between clinical cases of infection and the polymorphism of the selected genes.

The feature modeling, conducted using dominant effects and epistatic effects between genes, included 4 combinations of interactions (additive-additive, additive-dominant, dominant-additive, dominant-dominant). The effects were investigated depending on factors such as subsequent lactation, lactation stage, and somatic cell count (SCC).

As a result of the analysis carried out on the group of cows, polymorphic variants of *microRNA2313* and *microRNA2467* genes were identified, and monomorphism was found in *microRNA2489*, *microRNA29b-2*, and *microRNA29e*. The study demonstrated statistically significant associations between the polymorphism of the selected *microRNA2313* and *microRNA2467* genes and resistance/susceptibility to *mastitis* expressed as the number of somatic cells in milk. Importantly, the analysis showed the existence of dominant effects and interactions between genetic variants of the selected genes in shaping the variability of the somatic cell count in milk in the studied cow population and showed that the dominant and interactive effects of the studied alleles are an important element that allows for a comprehensive assessment of the overall effect of the allele.

A detailed analysis of the associations between the selected gene alleles and the number of somatic cells in milk in subsequent lactations, selected lactation stages, and SCC classes was carried out. However, at this stage of the research, it is difficult to unambiguously identify marker genes based on the obtained results, whose polymorphism could serve as a basis for conducting selection aimed at reducing the occurrence of *mastitis* without reducing milk yield and the concentration of components such as fat or protein in milk. However, the dominant effect of the T allele of the *microRNA2467* gene, which was associated with lower SCC content in milk in each lactation, presents a promising avenue that selection focused on increasing the frequency of the T allele of *microRNA2467* could yield satisfactory results in terms of reducing SCC content in milk in Holstein-Friesian cows.

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